Nitric oxide bioavailability and its potential relevance to the variation in susceptibility to the renal and vascular complications in patients with type 2 diabetes

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**Objective:** We compared the renal and systemic vascular (renovascular) response to reducing bioavailable nitric oxide in patients with type 2 diabetes without nephropathy of African and Caucasian heritage.

**Method:** Under euglycaemic conditions, renal blood flow was measured by a constant infusion of paraminohippurate and changes in blood pressure and renal vascular resistance estimated before and after an infusion of L-N⁶-monomethyl-l-arginine (L-NMMA).

**Results:** In the African heritage group there was a significant fall in renal blood flow ($\Delta - 46.0$ mls/min/1.73 m²; $p<0.05$) and rise in systolic blood pressure ($\Delta 10.0$ [2.3 – 17.9] mmHg; $p=0.017$) which correlated with an increase in renal vascular resistance ($r^2=0.77; p=0.004$).

**Conclusions:** The renal vasoconstrictive response associated with nitric oxide synthase inhibition in this study may be of relevance to the observed vulnerability to renal injury in patients of African heritage.
The bioavailability of nitric oxide (NO) is central to the regulation of renovascular function, and is reduced in established hypertension and diabetic nephropathy (1-3). Studies in rodents suggest that a deficiency of NO is an important susceptibility factor in the development of diabetes-related renal injury (4). It is unknown whether the differences in vulnerability to renal injury in patients with diabetes of African heritage (5) compared to Caucasians is related to NO bioactivity.

RESEARCH DESIGN AND METHODS

We studied patients with type 2 diabetes of African (AH) and Caucasian (CH) heritage. The patients in the AH (n=9) and CH (n=11) groups had a similar gender distribution, age and duration of diabetes (male 75 vs 70 %; p=0.89, 53.3 [7.2] vs 55.2 [4.6] years; p=0.50 and 10.3 [10.7] vs 6.8 [6.4] years; p=0.37). Systolic and diastolic blood pressure was 124.4 vs 122.1 mmHg; p=0.75 and 77.0 vs 76.1 mmHg; p=0.81 respectively. The patients were naïve to antihypertensive therapy and equal numbers in each group received metformin (n=6) and insulin (n=2).

Glycosylated haemoglobin (HbA1c) and urinary albumin were measured by high pressure liquid chromatography (HA 8121, Biomen, Berkshire, UK) and immunoturbidimetry respectively. Serum creatinine was analysed by a rate reaction method. Estimated creatinine clearance was calculated from the Cockcroft & Gault formula. Microalbuminuria was excluded on the basis of 3 consecutive albumin:creatinine (mg:mmol) ratios (ACRs) in sterile, early morning urine samples of < 3 mg/mmol, and a urinary albumin excretion rate of < 30 mg/day.

Renal plasma flow (RPF) was measured by the constant infusion method (6,7). A bolus dose of 8 mg/kg paraminohippurate (MSD, Hoddesdon, UK) was given with a 20 mg/min infusion. After a 90 minute equilibration period, the concentration of the infusate was multiplied by the infusion flow rate and divided by the mean of duplicate plasma samples at this, and subsequent time points. Plasma paraminohippurate was assayed after deproteinising the samples with 6% TCA for 10 min at 70° C and sequentially adding sodium nitrite, ammonium sulphamate and N-1-naphthylethylenediamine using a Cobas Mira (Roche, Lewes, UK).

After initial equilibration, an amino acid mixture (Vamin, Pharmacia & Upjohn, Milton Keynes, UK) was infused (0.043ml/kg/min). Renal plasma flow was assessed 80 minutes later and then L-NMMA (Clinalfa, Switzerland) was commenced at the ‘non-pressor’ dose of 20 µg/kg/min. Both infusions were continued for a further 20 minutes, after which a final RPF measurement was made.

During the studies, blood pressure was monitored automatically (Dinamap, Critikon, Basingstoke, UK) and whole blood was sampled from a venflon in a hand vein to measure glucose by the oxidase method (One Touch, Lifescan, High Wycombe, UK) every 10 minutes. Mean arterial pressure (MAP) was calculated as the diastolic blood pressure plus 1/3 of the pulse pressure. Renal blood flow (RBF) was calculated by dividing the RPF by (1-Hct) and renal vascular resistance (RVR) as MAP/RBF.

The study was approved by the ethics committee of the Whittington Hospital NHS Trust.

Statistics: Analyses between or within the groups were performed using SPSS 10.1 for Windows (Chicago, USA). Continuous variables were compared using parametric or non-parametric tests and associations tested with Spearman's rank or Pearson's test according to their distribution. Categorical variables were compared using Chi-squared
with continuity correction or Fisher's Exact test. Clearance and RPF measurements were corrected for a body surface area of 1.73m². Data are expressed as mean (standard deviation) unless otherwise stated.

RESULTS

Comparative baseline measurements of RPF, systolic and diastolic blood pressures were similar between the AH and CH groups (533.7[174.7] vs 565.3[260.8] mls/min/1.73m² [p=0.78], 124.9[23.7] vs 121.6[12.3] mmHg [p=0.29], and 77.1[9.5] vs 76.3[5.7] mHg; [p=0.81]). There were no differences in creatinine clearance or of the median [inter-quartile range] urinary albumin excretion rate (93.7[19.9] vs 98.9[19.5] mls/min/1.73m² [p=0.57] and (12.6 [4.1-25.0] vs 14.0[8.5-24.1] mg/day [p=0.79]). Averaged blood glucose was respectively similar (6.7[0.9] vs 7.4[0.9] mmol/l [p=0.14]). HbA1c was lower in the AH compared to the CH group (6.8[0.69] vs 8.0[0.94] % [p=0.005])

The L-NMMA infusion was associated with significant changes in systolic blood pressure in the AH group (Fig 1). Relative to the baseline and post amino acid measurements, there was a (mean [95% confidence interval]) rise of 10.0 [2.3 – 17.9] mmHg; p=0.017 and 7.3 [1.0-13.7] mmHg; p=0.03 in the AH group and 4.3[-1.8 to 10.4] mmHg;p=0.23 and 2.4[-3.5 to 8.3] mmHg ;p=0.38 in the CH group. Final blood pressure was higher in the AH group compared to the CH group (137.5[9.0] vs 123.4[14.2] mmHg [p<0.05]), and was associated with a fall in RBF (Δ -46.0 mls/min/1.73 m² ,p<0.05) and rise in RVR (from 0.12(0.06) to 0.14 (0.04) mmHg/ml/min/1.73m² ,[p=0.036]). The changes in RVR correlated with MAP (r²=0.77; p=0.004). Renal haemodynamic measures were unchanged in the CH group.

DISCUSSION

In this study, patients without hypertension or renal disease of African heritage, had an increased sensitivity to the renal vasconstrictive effect of nitric oxide synthase (NOS) inhibition. These data suggest that a reduction in NO bioavailability may adversely affect autoregulatory processes that could potentially increase the vulnerability to renal damage (8).

We used the amino acid infusion to optimize renal blood flow and suppress tubuloglomerular feedback as a contributor to vasoconstriction. The myogenic component of the autoregulatory response is attenuated by nitric oxide (9). Therefore, the reduction in renal blood flow we observed was probably due to an effect of NOS inhibition on the renovascular smooth muscle.

Early in the course of diabetes, NO production is necessary to forestall a rise in blood pressure. Hypertension is associated with the generation of NO-quenching free radicals and is a prerequisite for the development of renal disease (10-12). Furthermore, the renal expression of NOS in patients with diabetes is related to the degree of vasculopathy (13). It could therefore be considered, that an upregulation of NO production in patients of African heritage is related to a mechanism to oppose an enhanced vasoconstrictor tendency. Although consistent with experimental studies, these outcomes require caution before being generalised. Confirmatory studies in patients with and without diabetes with greater power, and the evaluation of the role of vasoconstrictive cytokines, angiotensin II and/or endothelin-1 as potential contributors to this haemodynamic response are now required.

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REFERENCES


Variable sensitivity to renal vasoconstriction by inhibition of NOS

A

![Graph showing renal plasma flow (RPF) with bars for Amino Acid and Amino Acid + L-NMMA with error bars and a p < 0.05 label.]  

B

![Graph showing systolic blood pressure (SBP) and diastolic blood pressure (DBP) over time with lines and error bars, and a p = 0.03 label.]